CLAIMS

1. A purified thermostable DNA polymerase having an amino acid sequence presented in SEQ ID NO: 2 from residue 1 to 776.

2. The polymerase of claim 1 that is isolated from *Thermococcus* species JDF-3.

3. The polymerase of claim 1 that is isolated from a recombinant organism transformed with a vector that codes for the expression of *Thermococcus* species JDF-3 DNA polymerase.

4. A recombinant vector comprising the nucleotide sequence presented in SEQ ID NO: 1.

5. An isolated recombinant polypeptide comprising the amino acid sequence of SEQ ID NO: 2.

6. An isolated recombinant DNA polymerase from *Thermococcus* species JDF-3 that is 3' to 5' exonuclease deficient.

7. The isolated recombinant DNA polymerase of claim 6, wherein said polymerase has a dual mutation comprising a serine to proline mutation at a site corresponding to S345 of SEQ ID NO: 2; and a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.

8. The isolated recombinant DNA polymerase of claim 6 that has an aspartic acid to threonine or alanine mutation at the amino acid corresponding to D141 of SEQ ID NO: 2 or a glutamic acid to alanine mutation at the amino acid corresponding to E143 of SEQ ID NO: 2.

9. The isolated recombinant DNA polymerase of claim 6 that has an aspartic acid to threonine or alanine mutation at the amino acid corresponding to D141 of SEQ ID NO: 2 and a glutamic acid to alanine mutation at the amino acid corresponding to E143 of SEQ ID NO: 2.

10. An isolated recombinant DNA polymerase having reduced discrimination against non-conventional nucleotides.

11. The DNA polymerase of claim 6 or 10 that is a Family B DNA polymerase.

12. The DNA polymerase of claim 6 or 10 wherein said DNA polymerase further comprises a mutation selected from the group consisting of: a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2; a leucine to phenylalanine mutation at a site corresponding to L408 of SEQ ID NO: 2; a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2; and an alanine to threonine mutation at a site corresponding to A485 of SEQ ID NO: 2.

13. The DNA polymerase of claim 12 wherein said polymerase having said alanine to threonine mutation at said site corresponding to A485 of SEQ ID NO:2 further comprises a mutation selected from the group consisting of: a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2; a leucine to phenylalanine mutation at a site corresponding to L408 of SEQ ID NO: 2; a serine to proline mutation at a site corresponding to

S345 of SEQ ID NO: 2; and a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.

14. The DNA polymerase of claim 6 or 10 that has reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

15. The DNA polymerase of claim 14 wherein said conjugated nucleotide is selected from the group consisting of radiolabeled nucleotides, fluorescently labeled nucleotides, biotin labeled nucleotides, chemiluminescently labeled nucleotides and quantum dot labeled nucleotides.

16. An isolated recombinant Family B DNA polymerase comprising an alanine to threonine mutation at the site corresponding to A485 of SEQ ID NO: 2, or a mutation at a site corresponding to L408, S345 or P410 of SEQ ID NO: 2, wherein said DNA polymerase has reduced discrimination against non-conventional nucleotides relative to the wild-type form of that polymerase.

17. The polymerase of claim 16 that is 3' to 5' exonuclease deficient.

18. The polymerase of claim 17 that has a mutation at an amino acid corresponding to D141 or E143 of SEQ ID NO: 2.

19. The polymerase of claim 17 that has an aspartic acid to threonine or alanine mutation at a site corresponding to D141 of SEQ ID NO: 2.

20. The polymerase of claim 17 that has a glutamic acid to alanine mutation at a site corresponding to E143 of SEQ ID NO: 2.

21. The polymerase of claim 20 that has an aspartic acid to threonine or alanine mutation at the amino acid corresponding to D141 of SEQ ID NO: 2.

22. The polymerase of claim 16 that is thermostable.

23. The polymerase of claim 16 that is archaeal.

24. The polymerase of claim 16 wherein said DNA polymerase comprises a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2.

25. The polymerase of claim 16 wherein said DNA polymerase comprises a leucine to phenylalanine mutation at a site corresponding to L408 of SEQ ID NO: 2.

26. The polymerase of claim 16 wherein said DNA polymerase comprises a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.

27. The polymerase of claim 16 wherein said DNA polymerase comprises a serine to proline mutation at a site corresponding to S345 of SEQ ID NO: 2, wherein said polymerase may further comprise a mutation at a site corresponding to T604 of SEQ ID NO: 2.

28. The polymerase of claim 16, wherein said DNA polymerase comprises a tyrosine to cysteine mutation at a site corresponding to Y497 of SEQ ID NO: 2, wherein said polyerase may further comprise an isoleucine to valine mutation at a site corresponding to I630 of SEQ ID NO: 2.

29. The polymerase of claim 16, wherein said DNA polymerase comprises a glutamic acid to lysine mutation at a site corresponding to E645 of SEQ ID NO: 2.

30. The polymerase of claim 16, wherein said DNA polymerase comprises a glutamic acid to lysine mutation at a site corresponding to E578 of SEQ ID NO: 2, wherein said polymerase may further comprise an arginine to methionine mutation at a site corresponding to R465 of SEQ ID NO: 2.

31. The polymerase of claim 16, wherein said DNA polymerase comprises a leucine to glutamine mutation at a site corresponding to L396 of SEQ ID NO: 2, wherein said polymerase further comprises a mutation at a site corresponding to V401, N424, P569, E617, or V640 of SEQ ID NO: 2.

32. The polymerase of claim 16, wherein said DNA polymerase comprises a serine to asparagene mutation at a site corresponding to S651 of SEQ ID NO: 2.

33. The polymerase of claim 16, wherein said DNA polymerase comprises a leucine to proline mutation at a site corresponding to L396 of SEQ ID NO: 2, wherein said polymerase may further comprise a mutation at a site corresponding to E459 of SEQ ID NO: 2.

34. The polymerase of claim 16, wherein said DNA polymerase comprises a leucine to proline mutation at a site corresponding to L456 of SEQ ID NO: 2, wherein said polymerase may further comprise a mutation at a site corresponding to E658 of SEQ ID NO: 2.

35. The polymerase of claim 16, wherein said DNA polymerase comprises a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2, wherein said polymerase may further comprise a mutation at a site corresponding to V437, or L478 of SEQ ID NO: 2.

36. The polymerase of claim 16 wherein said DNA polymerase comprises an tyrosine to asparagine mutation at a site corresponding to Y496 of SEQ ID NO: 2.

37. The polymerase of claim 16 wherein said DNA polymerase comprises an alanine to threonine mutation at a site corresponding to A485 of SEQ ID NO: 2.

38. The polymerase of claim 37 comprising a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2.

39. The polymerase of claim 37 comprising a leucine to phenylalanine mutation at a site corresponding to L408 of SEQ ID NO: 2.

40. The polymerase of claim 37 comprising a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.

41. The polymerase of claim 16, having reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

42. The polymerase of claim 41 wherein said conjugated nucleotide is selected from the group consisting of radiolabeled nucleotides, fluorescently labeled nucleotides, biotin labeled nucleotides, chemiluminescently labeled nucleotides and quantum dot labeled nucleotides.

43. The polymerase of claim 10 or 16 further comprising a mutation at an amino acid residue in the polymerase that corresponds to a mutation selected from the group consisting of: a Y to V mutation at amino acid 409 of SEQ ID NO:2; an A to C, S, L, I, F, or V mutation at amino acid 485 of SEQ ID NO: 2; a Y to S mutation at amino acid 494 of SEQ ID NO: 2; a Y to L mutation at amino acid 496 of SEQ ID NO: 2; and an A to Y mutation at amino acid 490 of SEQ ID NO: 2.

44. The polymerase of claim 10 or 16 further comprising a mutation at an amino acid of the polymerase corresponding to one of amino acids 483 to 496, inclusive, of SEQ ID NO: 2.

45. The polymerase of claim 44 wherein said mutation is at an amino acid of the polymerase corresponding to one of amino acids 485, 490, 494, or 496 of SEQ ID NO: 2.

46. An isolated recombinant Family B DNA polymerase comprising an alanine to threonine mutation at an amino acid corresponding to A485T of SEQ ID NO: 2 and and at least one substitution in the polymerase of an amino acid corresponding to L408, Y409, S345 or P410 respectively, of SEQ ID NO: 2.

47. An isolated recombinant Family B DNA polymerase comprising an amino acid other than A at an amino acid of the polymerase corresponding to A485 of SEQ ID NO: 2, and at least one substitution in the polymerase of an amino acid corresponding to L408, Y409, S345 or P410, respectively, of SEQ ID NO: 2.

48. A recombinant vector comprising a nucleic acid sequence encoding the DNA polymerase of any one of claims 1, 5, 6, 10, 16, 46 or 47.

49. A method of labeling a complementary strand of DNA, which method comprises the step of:

contacting a template DNA molecule with:

a recombinant Family B DNA polymerase of claim 10; and

a non-conventional nucleotide under conditions and for a time sufficient to permit said DNA polymerase to synthesize a complementary DNA strand and to incorporate said non-conventional nucleotide into said complementary DNA strand.

50. A method of labeling a complementary strand of DNA, which method comprises the step of:

contacting a template DNA molecule with:

a recombinant Family B DNA polymerase comprising an alanine to threonine mutation at a site corresponding to A485 of SEQ ID NO: 2 or a mutation at a site corresponding to L408 or P410 or S345 of SEQ ID NO: 2, wherein said DNA polymerase has reduced discrimination against non-conventional nucleotides; and

a non-conventional nucleotide under conditions and for a time sufficient to permit said DNA polymerase to synthesize a complementary DNA strand and to incorporate said non-conventional nucleotide into said complementary DNA strand.

51. The method of claim 50 wherein said recombinant Family B DNA polymerase is 3'-5' exonuclease deficient.

52. The method of any one of claims 49-51 herein said recombinant Family B polymerase comprises a leucine to histidine mutation at a site corresponding to amino acid L408 of SEQ ID NO: 2.

53. The method of any one of claims 49-51 wherein said recombinant Family B polymerase comprises a leucine to phenylalanine mutation at a site corresponding to amino acid L408 of SEQ ID NO: 2.

54. The method of any one of claims 49-51 wherein said recombinant Family B polymerase comprises a proline to leucine mutation at a site corresponding to amino acid P410 of SEQ ID NO: 2.

55. The method of any one of claims 49-51 wherein said recombinant Family B polymerase comprises an alanine to threonine mutation at a site corresponding to amino acid A485 of SEQ ID NO: 2.

56. The method of claim 55 wherein said recombinant Family B polymerase comprises a leucine to histidine mutation at an amino acid corresponding to L408 of SEQ ID NO: 2.

57. The method of claim 55 wherein said recombinant Family B polymerase comprises a leucine to phenylalanine mutation at an amino acid corresponding to L408 of SEQ ID NO: 2.

58. The method of claim 55 wherein said recombinant Family B polymerase comprises a proline to leucine mutation at an amino acid corresponding to P410 of SEQ ID NO: 2.

59. The method of claim 55 wherein said recombinant Family B polymerase comprises a serine to proline mutation at an amino acid corresponding to S345 of SEQ ID NO: 2.

60. The method of claim 55, wherein said recombinant Family B polymerase has a dual mutation comprising a serine to proline mutation at a site corresponding to S345 of SEQ ID NO: 2; and a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.

61. The method of any one of claims 49-51 wherein said recombinant Family B polymerase has reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides, and conjugated nucleotides.

62. The method of claim 61 wherein said conjugated nucleotide is selected from the group consisting of radiolabeled nucleotides, fluorescently labeled nucleotides, biotin labeled nucleotides, chemiluminescently labeled nucleotides and quantum dot labeled nucleotides.

63. A method of sequencing DNA comprising the steps of:

contacting a template DNA strand with:

a sequencing primer;

a recombinant Family B DNA polymerase of claim 9; and

a chain-terminating nucleotide analog, under conditions that permit said DNA polymerase to synthesize a complementary DNA strand and to incorporate nucleotides into the synthesized complementary DNA strand, wherein incorporation of a chain-terminating nucleotide analog results in the termination of chain elongation, such that the nucleotide sequence of said DNA strand is determined.

64. A method of sequencing DNA comprising the steps of:

contacting a DNA strand with:

a sequencing primer;

a recombinant Family B DNA polymerase comprising an alanine to threonine mutation at a site corresponding to A485 of SEQ ID NO: 2 or a mutation at a site corresponding to L408 or P410 or S345 of SEQ ID NO: 2, wherein said DNA polymerase has reduced discrimination against non-conventional nucleotides; and

a chain-terminating nucleotide analog, under conditions that permit said DNA polymerase to synthesize a complementary DNA strand and to incorporate nucleotides into the synthesized complementary DNA strand, wherein incorporation of a chain-terminating nucleotide analog results in the termination of chain elongation, such that the nucleotide sequence of said DNA strand is determined.

65. The method of claim 64 wherein said recombinant Family B polymerase is deficient in 3' to 5' exonuclease activity.

66. The method of any one of claims 63-65 wherein said recombinant Family B polymerase has a leucine to histidine mutation at a site corresponding to amino acid L408 of SEQ ID NO: 2.

67. The method of any one of claims 63-65 wherein said recombinant Family B polymerase has a leucine to phenylalanine mutation at a site corresponding to amino acid L408 of SEQ ID NO: 2.

68. The method of any one of claims 63-65 wherein said recombinant Family B polymerase has a proline to leucine mutation at a site corresponding to amino acid P410 of SEQ ID NO: 2.

69. The method of any one of claims 63-65 wherein said recombinant Family B polymerase has an alanine to threonine mutation at a site corresponding to amino acid A485 of SEQ ID NO: 2.

70. The method of claim 69 wherein said recombinant Family B polymerase has a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2.

71. The method of claim 69 wherein said recombinant Family B polymerase has a leucine to phenylalanine mutation at a site corresponding to L408 of SEQ ID NO: 2.

72. The method of claim 69 wherein said recombinant Family B polymerase has a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.

73. The method of claim 69 wherein said recombinant Family B polymerase has a serine to proline mutation at a site corresponding to S345 of SEQ ID NO: 2.

74. The method of any one of claims 63-65 wherein said chain-terminating nucleotide analog is a dideoxynucleotide.

75. The method of claim 74 wherein said dideoxynucleotide is detectably labeled.

76. The method of claim 75 wherein said dideoxynucleotide is fluorescently labeled.

77. The method of claim 76 wherein said dideoxynucleotide is labeled with a moiety selected from the group consisting of fluorescein and rhodamine.

78. A kit for performing the method of claim 49, 50, 51, 63, or 64.

79. A method of making a purified thermostable DNA polymerase having an amino acid sequence presented in SEQ ID NO: 2 from residue 1 to 776, comprising culturing a host cell containing the nucleic acid sequence presented in SEQ ID NO:1 under conditions which permit production of said DNA polymerase.

80. A method of making a recombinant DNA polymerase of *Thermococcus* species JDF-3 that is 3' to 5' exonuclease deficient, comprising culturing a host cell containing a nucleic acid sequence encoding said polymerase under conditions which permit production of said DNA polymerase.

81. A method of making a recombinant DNA polymerase having reduced discrimination against non-conventional nucleotides, comprising culturing a host cell containing a nucleic acid sequence encoding said polymerase under conditions which permit production of said DNA polymerase.

82. A method of making a recombinant Family B DNA polymerase comprising an alanine to threonine mutation at the site corresponding to A485 of SEQ ID NO: 2 or a mutation at a site corresponding to L408 or P410 or S345 of SEQ ID NO: 2, wherein said DNA polymerase has reduced discrimination against non-conventional nucleotides relative to the wild-type form of

that polymerase, comprising culturing a host cell containing a nucleic acid sequence encoding said polymerase under conditions which permit production of said DNA polymerase.

83. The method of claim 79, ~~80, 81~~, or 82, wherein said host cell is *E. Coli*.

84. The method of claim 79, 80, 81, or 82, wherein said host cell is *Thermococcus*.

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